STUDIES IN THE PHYSICAL CHEMISTRY OF AMINO ACIDS AND PROTEINS

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By

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Electrostriction of Amino Acid Solution

It is well known that amino acids and proteins have various polar groups such as COO^-, NH_3^+, OH, CONH. etc. and form zwitterion in water solution. Among these polar groups the ionized carboxyl-and amino-groups are the most important to the hydration of amino acids and proteins. Cohn et al. (1) have determined the apparent molal volume of amino acids and related compounds and then calculated the electrostriction of solvent. They did not, however, distinguish between the electrostriction due to carboxyl group and that of amino group. In present work the author has determined the electrostriction of solvent due to each polar groups and observed that the amino group hydrates more than the carboxyl group.

A. *Calculation of the Electrostriction of the Solvent due to the Polar Groups of Amino Acids.*

In general the apparent molal volume φ of solute is represented as

$$\varphi = \frac{(V - n_1 v_1)}{n_2} ,$$

in which V is the volume of solution, v_1 is the molal volume of the solvent, n_1 and n_2 are the number of mols of solvent and solute respectively. Traube (2) showed that the apparent molal volume of organic compounds could be represented as the total sum of the apparent atomic volume of each constitutional atom. But in the case of simple ions and amino acids, the apparent molal volume are always less than that obtained according to Traube's low.

This is accounted for by the fact that the polar molecules of water are attracted to the ion or polar group and there occurs remarkable contraction

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of volume of the solvent. Such a volume contraction of the solvent is called "Electrostriction". The author has calculated the electrostrictions of solvent due to the charged carboxyl-and amino-groups of amino acids by the following method.

The apparent molal volume of amino acid zwitterion ($R^{\pm}$) is directly calculated from the density of isoelectric solution of amino acids. By adding equimolecules of HCl into this solution, the greater part of zwitterions becomes amino acid anion (R^{+}), namely,

$$\underset{x}{R\text{-}\underset{NH_3^+}{CH}\text{-}COO^-} + \underset{x}{H^+} + \underset{x}{Cl^-} \rightarrow \underset{m-x}{R\text{-}\underset{NH_3^+}{CH}\text{-}COOH} + Cl^-.$$

Assuming (m) is the total concentration of amino acids, x is the concentration of amino acid zwitterions and K_1 is the apparent ionization constant of carboxyl group, we may write

$$K_1 = \frac{[R^{\pm}][H^+]}{[R^+]} = \frac{x^2}{m-x} \quad . \tag{1}$$

Then the apparent molal volume of amino acid cation is represented as follows:

$$\varphi_{R^+} = \frac{1}{m-x}\left\{ m(\Phi_1 - \varphi_{Cl^-}) - x(\varphi_{R^{\pm}} + \varphi_{H^+}) \right\}, \tag{2}$$

in which Φ_1, φ_{H^+} and φ_{Cl^-} are the apparent molal volume of total solute, hydrogen ion and chlorine ion respectively. By the same principle the apparent molal volume of amino acid cation is calculated from the following equations:

$$K_2 = \frac{x^2}{m-x} \tag{3}$$

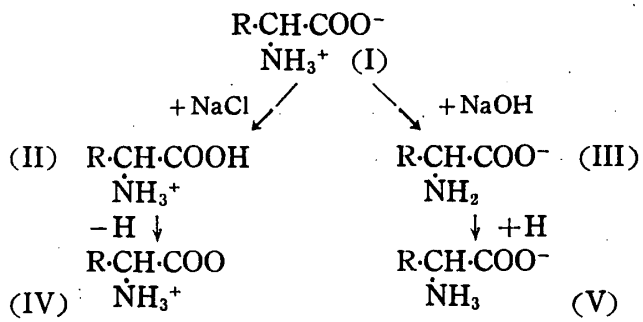
$$\varphi_{R^-} = \frac{1}{m-x}\left\{ m(\Phi_2 - \varphi_{Na^+}) - x(\varphi_{R^{\pm}} + \varphi_{OH^-}) \right\}, \tag{4}$$

in which K_2 is the ionization constant of amino group, Φ_2, φ_{Na^+} and φ_{OH^-} are the apparent molal volume of total solute, sodium ion and hydroxyl ion respectively.

Let us now substract the hydrogen atom from the carboxyl group of amino acid cation, then we obtain a hypothetic form of amino acid cation IV.

The difference between I and IV lies in that the carboxyl group of I has the negative charge but that of IV has no charge. Thus the difference between the φ values of I and IV shows the electrostriction of solvent due to the charged carboxyl group.

In the same way the hypothetic form of the amino acid anion is considered and the following calculation used to obtain the electrostriction of the solvent due to the charged amino group.



B. *Experimental*

The apparent molal volumes of glycine, DL-alanine, DL-α-amino-n-butyric acid, α-amino-isobutyric acid, DL-α-amino-n-valeric acid, DL-valine, L-leucine, β-alanine, δ-amino valeric acid, ε-amino caproic acid have been measured and the electrostriction of the solvent due to the charged carboxy-and amino-groups were calculated. The results are summerized in Table I.

Table I. Apparent Molal Volumes of Amino Acid Ions and Electrostrictions of the Solvent (Temp. 25.00±0.05°C, 0.25 m/1000 g H_2O)

Amino acid	App. mol. vol. of amino acid			Electrostriction	
	Zwitterion $\varphi_{R\pm}$	Cation φ_{R+}	Anion φ_{R-}	$-COO^-$	$-NH_3^+$
Glycine	43.8 cc.	49.0 cc.	48.9 cc.	2.1 cc.	8.2 cc.
DL-Alanine	60.1	67.0	63.8	3.8	6.8
DL-α-amino-n-butyric acid	76.0	83.1	79.9	3.9	7.0
DL-α-amino-n-valeric acid	92.4	99.2	96.4	3.7	7.1
α-amino-isobutyric acid	76.7	83.5	79.1	3.7	5.5
DL-Valine	91.3	98.4	93.4	4.0	5.2
L-Leucine	107.3	114.2	111.1	3.8	6.9
β-Alanine	58.1	64.1	63.1	3.0	8.1
δ-amino-valeric acid	89.7	98.5	94.7	5.7	8.1
ε-amino-caproic acid	104.6	114.7	109.8	7.0	8.3

From this Table we can draw the following conclusions;

(1) The electrostrictions due to charged amino groups of α-amino acids are greater than those of charged carboxyl groups.

(2) The electrostrictions due to carboxyl groups of α-amino acids show approximately a constant value of 3.8 cc./mol., but that of amino groups, on the other hand, is affected by the steric structure of R-groups of amino acids.

(3) The magnitude of the electrostriction by charged amino groups of amino acids, such as glycine, β-alanine, δ-aminovaleric acid and etc. are

8.1-8 3 cc./mol. This suggests that the terminal amino groups have almost the same effect on the volume contraction.

The free energy of hydration of glycine

The free energy of hydration of glycine was estimated from the equation which shows the relationship between the partial molal volume of glycine and the dielectric constant of a glycine solution.

Assuming the amino acid zwitterion to be spherical, and choosing as the standard state, a solution of the zwitterion at low concentration in a solvent of dielectric constant D', Kirkwood (3) showed the following equation as the expression for the activity coefficient in a solvent of dielectric constant D.

$$\log \gamma = \frac{1}{2kT} \sum_{n=0}^{\infty} \frac{(n+1)Qn}{a^{2n+1}} \left\{ \frac{1-D}{(n+1)D+n} - \frac{1-D_0}{(n+1)D_0+n} \right\} \tag{5}$$

k ····· Boltzmann's constant

T ····· Absolute temperature

n ····· 0, 1, 2, 3, ·····

Qn ····· The early numbers of the function Qn have the form

$$Q_0 = 0$$
$$Q_1 = \mu^2$$
$$Q_2 = 3\mu^2 \left(r^2 - \frac{R^2}{4}\right)$$
$$\vdots$$

in which μ is the dipole moment, R is the distance between the center of charge and the center of zwitterion, R is the distance between the centers of negative charge e^- and positive charge e^+. In the case of glycine it is enough to take the term up to n=1 for the first approximation.

In general the free energy of hydration may be considered as the difference of potential energy between in a vacuum ($D = 1$) and in a solvent. Since the charging work in solution is

$$W = \log \gamma \, kT,$$

the free energy of hydration ΔF in a very dilute solution can be represented as follows:

$$\Delta F = \bar{F}^0 - F\bar{v}^0 = -N\frac{\mu^2}{a^3}\left(\frac{D-1}{2D+1}\right), \tag{6}$$

in which $\bar{F}_0$ is the partial molal free energy, $F\bar{v}^0$ is molal free energy in a vacuum, N is Avogadro's number, μ is the dipole moment of glycine molecule and the molecular radius of glycine.

By differentiating equation 2 with respect to pressure at constant tem-

perature and using the thermodynamical equation

$$\left(\frac{\partial F}{\partial P}\right)_T = V,$$

find $$\Delta V = \bar{V}^0 - V_v{}^0 = -\frac{3N\mu^2}{a^3} \cdot \frac{1}{(2D+1)^2} \cdot \frac{\partial D}{\partial P}. \qquad (7)$$

Determinating experimentally the relationship between the partial molal volume of the solute and the dielectric constant of the solvent and combining these results with equations 6 and 7, we can obtain the free energy of hydration.

Experimental

The apparent molal volumes of glycine in acetone-water solution were measured by the usual method. The results are set forth in Table II.

Table II. Apparent Molal Volumes of Glycine in Acetone-water Solution (Temp. 12.00±0.05°C)

Water, d=0.99952			10% acetone, d=0.98758		
Concentration of glycine	Density	App. mol. vol.	Concentration of glycine	Density	App. mol. vol.
0.0995	1.00278	42.51 cc.	0.0984	0.99084	41.93
0.1980	1.00590	42.63	0.1961	0.99405	42.27
0.2961	1.00911	42.73	0.2926	0.99739	42.56
5% acetone, d=0.99656			15% acetone, d=0.98188		
0.0989	0.99666	42.27	0.0978	0.98524	41.47
0.1970	0.99986	42.45	0.1948	0.98848	41.97
0.2912	1.00291	42.66	0.2909	0.99161	42.38

Extrapolating to infinity and plotting the apparent molal volume of glycine at infinite dilution against the dielectric constant of the solvent, we have obtained the results shown in Figure 1. From these results and equation 3, we may calculate the value of $\frac{\mu^2}{a^3}$, namely,

$$\frac{\mu^2}{a^3} = 14.49 \times 10^{-12} \ c.g.s., \qquad (8)$$

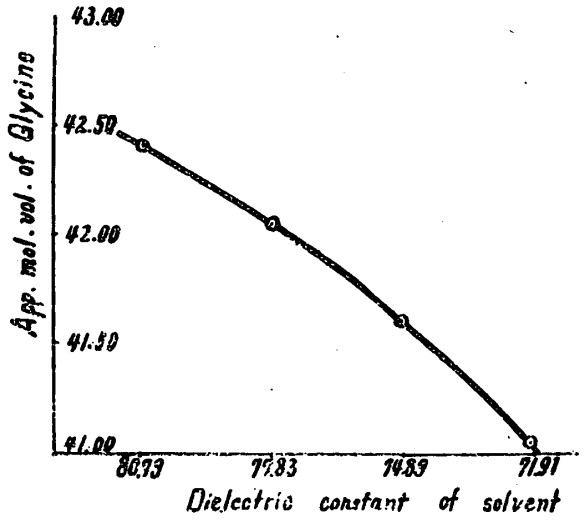
In the calculation necessary date were cited from the Owen's paper (4)

$$\frac{\partial D}{\partial P} \ of \ water = 48.05 \times 10^{-10} \ c.g.s., \quad D = 80.79$$

$$\frac{\partial D}{\partial P} \ of \ acetone = 30.63 \times 10^{-10} \ c.g.s., \quad D = 21.50$$

Combinating the above value of $\frac{\mu^2}{a^3}$ and equation 2, we find the value for the free energy of hydration of glycine to be

$$\Delta F = -102.3K \; cal/mol.$$



Fig, 1.

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